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14. ABSTRACT

Although the rate of advances in prostate cancer research is rapidly accelerating, there still remains an urgent need for development of more effective therapy for castrate resistant metastatic prostate cancer (CRPC). Based upon a substantial published literature from multiple groups, as well as unpublished studies to be presented from the applicants laboratories, an exciting approach that has not been tested clinically involves isolating a specific type of healthy bone marrow derived cells and loading it with a therapeutic chemical so that when these loaded cells are injected into the blood stream, they are selectively retained (i.e., "home") to metastatic sites of cancer in castration resistant metastatic prostate cancer patients. The therapeutic chemical delivered via these injected cells is selectively engineered to act like a "molecular grenade" in that it is designed to "detonate" upon release of a non-selective toxin restrictively within the microenvironment of metastatic sites of cancer. This approach is both exciting and practical because the cells used for this selective cancer delivery of the molecular grenade can be routinely harvested from healthy bone marrow donors and do not need to be host matched and have been safely infused into humans to treat other non-cancer diseases. To develop such a cell based molecular grenade delivery as systemic therapy for metastatic CRPC, a multi-disciplinary/multi-institutional/multi-investigator team has been assembled based upon the synergistic (i.e., team) expertise needed to translating the basic science discoveries concerning cancer homing into clinical trials for CRPC.

15. SUBJECT TERMS
Trojan horse therapy, prostate cancer homing, allogeneic bone marrow, cell based therapeutics

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Attacking prostate cancer with a prodrug-doped cellular Trojan horse

Table of Contents

Page

1. Introduction

Prostate cancer (PCa) is the second most common cancer and the second leading cause of cancer-related deaths in American men. Currently affecting over 2.5 million Americans, 1 in 7 men in the U.S. will be diagnosed with PCa in their lifetime[1]. PCa lethality is fueled by the development of disseminated metastases, which are commonly found in the bone, lymph nodes, liver and lungs[2-4]. Despite the impressive progress in PCa research and the availability of therapies such as surgery, radiation, hormonal therapy, immunotherapy and chemotherapy, there remains a significant need for more effective therapies for castration-resistant metastatic PCa[2,4-8]. Specifically, there is a need to efficiently target systemically administered anti-cancer drugs to sites of PCa metastasis while minimizing host toxicity. Systemic administration of therapeutic agents typically encounters multiple challenges including severe adverse effects due to systemic toxicity, uncontrolled drug levels, premature enzymatic/chemical inactivation and rapid drug clearance requiring repeated dosing[9]. While drug encapsulation in nano/micro delivery systems may reduce host toxicity and protect the drug from early degradation, effective targeting of tumors remains elusive[10]. Moreover, systemically infused micro/nanoparticles typically remain close to blood vessels and cannot efficiently distribute the drug throughout the tumor [11–14].

A potential approach to overcome such challenges is to use a cell-based platform for targeted delivery of therapeutics to sites of metastatic PCa. Known to display tropism towards cancer sites[15-17], mesenchymal stem cells (MSCs) are potential candidates. One advantage of using particle-loaded cells is that they might migrate away from the vasculature and deeper into the tumor to effectively distribute their toxic payload throughout the tumor. Furthermore, allogeneic MSCs can be harvested from the bone marrow of healthy donors and expanded *ex-vivo* using well-established, FDA-approved protocols[18]. Displaying immune evasiveness, these allogeneic MSCs do not need to be host matched, providing yet another advantage towards their clinical translation[19]. Indeed, MSCs are being explored in over 500 clinical trials worldwide. Clinical studies have demonstrated that hundreds of millions of allogeneic MSCs can be safely administered intravenously (IV) without significant side effects[19].

To further reduce host toxicity and provide yet another layer of specificity to our delivery system, we chose to use the macromolecule G114, a thapsigargin-based Prostate Specific Antigen (PSA)-activated prodrug[20-23]. PSA is a serine protease that is only secreted by prostate epithelial cells[24-27]. Although PSA is detected in the blood of PCa patients, it is enzymatically inactive due to binding with ubiquitous serum protease inhibitors such as alpha-1-antichymotrypsin (α1-AC) and alpha-2-macroglobulin (α2M)[25]. Importantly, the enzymatically active form of PSA is only present in the extracellular fluid (ECF) within the prostate and sites of PCa including metastases[25]. We have previously engineered G114, a cell-impermeable PSA-activated prodrug comprised of the potent cytotoxic molecule leucine-12-aminododecanoyl thapsigargin (Leu-12ADT), an amino acid-thapsigargin analog, conjugated to a unique, PSA-cleavable, five amino acid peptide, HSSKLQ[20-23]. Unproteolyzed G114 is inactive and cannot penetrate cells until it reaches PCa sites, where it is cleaved by PSA to liberate the active toxin, Leu-12ADT. The released lipophilic toxin rapidly enters adjacent cells and induces apoptosis in a proliferation-independent manner via inhibition of the sarcoplasmic/endoplasmic calcium ATPase (SERCA) pump[28-30]. Therefore, G114 represents a highly selective, PSA-targeted agent with demonstrated anti-tumor efficacy in preclinical models of PCa. Unfortunately, G114, like other peptide-based prodrugs, suffers from unfavorable pharmacokinetics with a plasma half-life of only a few hours due to renal clearance. Thus, G114 is a suitable candidate for our particle-in-cell delivery platform.

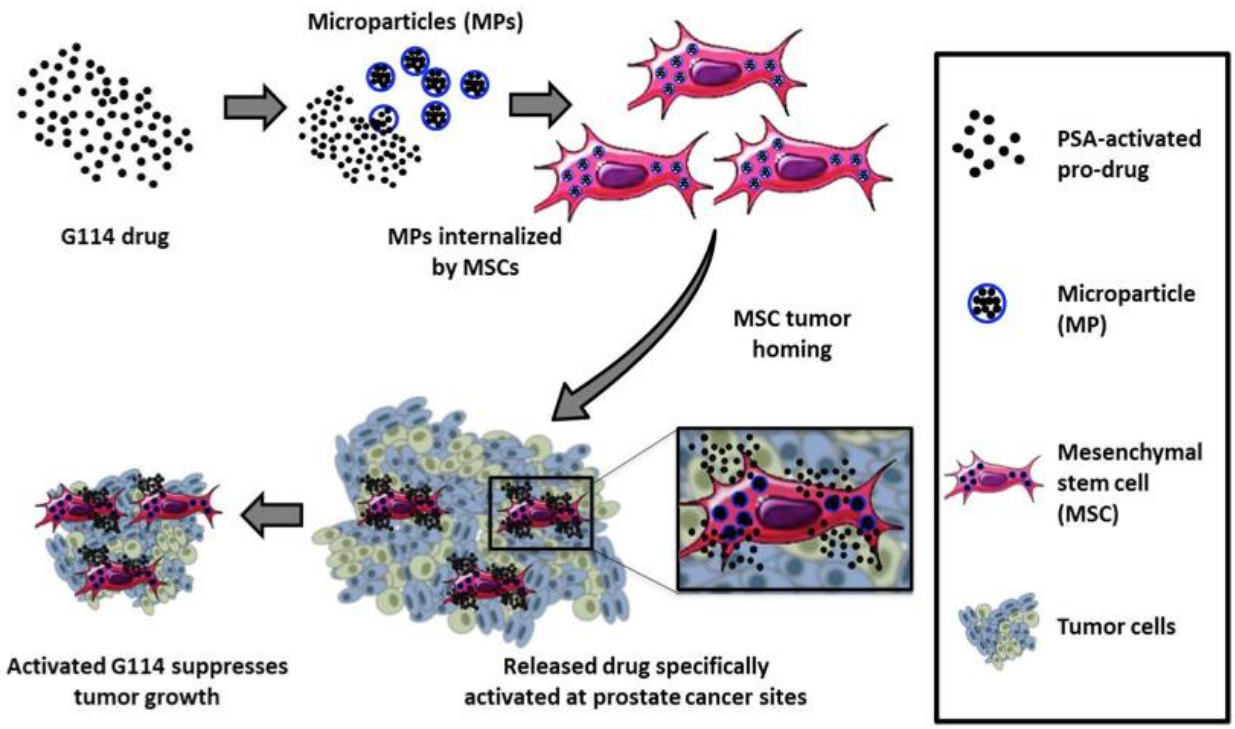


Figure 1: Mesenchymal stem cells doped with drug-loaded PLGA microparticles as a potential cell-based treatment for prostate cancer.

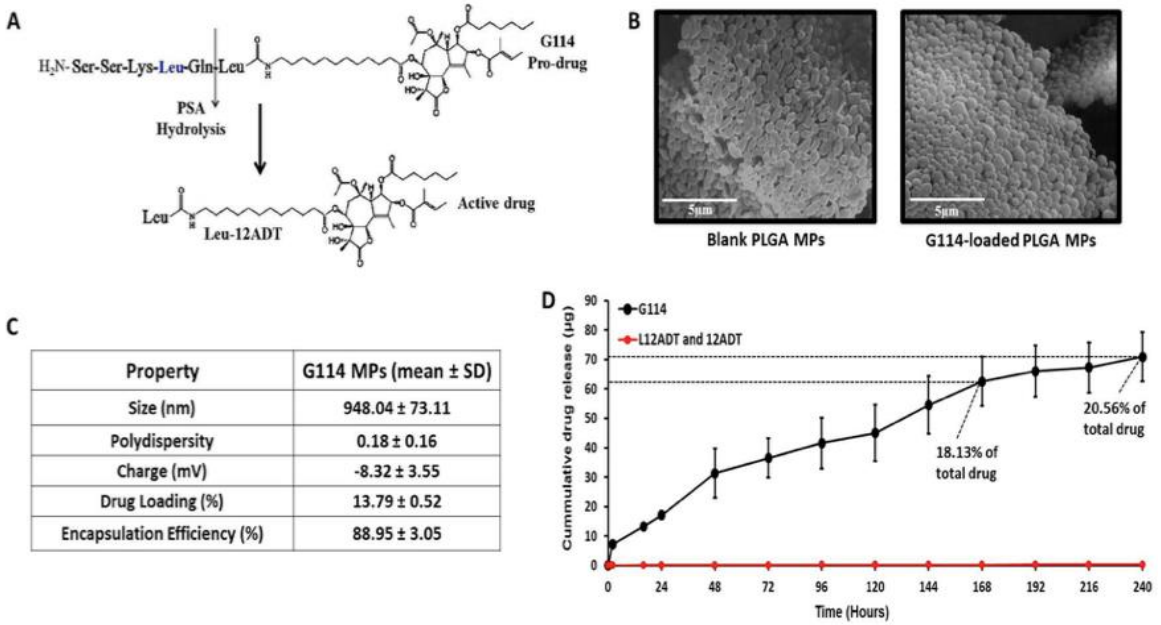
In this proof-of-concept study, we aimed to develop engineered MSCs as a G114 delivery platform for PCa therapy **(Figure 1)**. First, we sought to encapsulate G114 in poly(lactic-co-glycolic acid) microparticles (PLGA MPs). PLGA is already present in FDA-approved products; it is a biodegradable, biocompatible polymer that enables tunable drug release [31–33] and we have previously used PLGA MPs to encapsulate small molecules to control cell phenotype[34-36]. Following an intricate iteration of a double emulsion protocol[37-38], we succeeded in encapsulating G114, a peptide-containing prodrug (M.W. >1600g/mole), in PLGA particles (~950nm in diameter), achieving high drug loading (>13%) and encapsulation efficiency (>88%). G114 MPs were then successfully internalized by MSCs without compromising their viability. G114 was released as a functional, intact prodrug in significant levels from G114-MP-loaded MSCs for up to 7 days and selectively induced cell death of PSA-secreting PCa cells *in-vitro*. Finally, co-inoculation *in-vivo* studies demonstrated the therapeutic efficacy of G114-MP-loaded MSCs, suggesting that cell-based delivery of G114 did not compromise the potency of this pro-drug *in-vitro* or *in-vivo*. Overall, this study highlights the potential of G114-MP-loaded MSCs as cell-based delivery vehicles for PCa therapy. Furthermore, achieving efficient targeting of systemically infused MSCs to sites of PCa metastasis may facilitate the development of such drug-loaded cells into a potent systemic therapy for metastatic PCa.

2. Keywords

Mesenchymal stem cells, targeted drug delivery, poly(lactic-co-glycolic acid) (PLGA), PLGA microparticles (MPs), leucine-12-aminododecanoyl thapsigargin (Leu-12ADT), cell-based delivery, cell therapy, prodrug therapy, enzyme-based cleavage, prostate-specific antigen (PSA)

3. Overall Project Summary

Despite rapid progress in PCa therapy, there is still a major need to develop systemic treatments that can effectively target sites of metastatic PCa, which is known to preferentially metastasize to the bone[2]. Standard chemotherapies, while effectively killing cancer cells, also result in adverse side effects [39,40]. Encapsulation of cytotoxic agents in micro/nanoparticles has emerged over the last two decades as a promising approach to overcome some of the challenges in systemic delivery of such therapeutics[10,41]. Indeed, micro/nanoparticle delivery systems can protect drugs from the harsh *in-vivo* environment post-infusion, improve drug delivery and were also shown in some cases to augment therapeutic efficacy and reduce systemic toxicity[10,42-43]. Nevertheless, targeting particles to cancer sites, and especially the bone marrow, remains challenging. MSCs in particular represent a promising vector for this approach because they are known to be well tolerated by patients[19] and to display cancer tropism[15-17]. Hence, the use of cellular carriers of microparticles represents a promising strategy for targeted delivery of anti-cancer drugs to cancer sites [44-47]. **(SOW Task 1)** A Phase 0 pre-prostatectomy clinical trial (NCT01983709) is on-going, designed to quantify the number of systemically infused allogeneic human bone marrow-derived MSCs to sites of PCa. This study will provide the baseline conditions for the eventual clinical translation of the particle-in-a-cell platform. **(SOW Task 2, subtasks 1-5)** We have developed a cell-based platform using MSCs loaded with microparticles (MPs) containing a PSA-specific produg, G114, that can be delivered to and kill prostate cancer cells. G114 is composed of a thapsigargin-based toxin and a PSA-cleavable peptide sequence (i.e. HSSKLQ), which confers its specificity **(Figure 2A)**. First, using a double emulsion protocol[11], G114 was successfully encapsulated in PLGA MPs, with optimized drug loading of app. 13% and encapsulation efficiency of app. 88%, indicative of an efficient encapsulation process **(Figure 2B)**.



Property	G114 MPs (mean ± SD)
Size (nm)	948.04 ± 73.11
Polydispersity	0.18 ± 0.16
Charge (mV)	-8.32 ± 3.55
Drug Loading (%)	13.79 ± 0.52
Encapsulation Efficiency (%)	88.95 ± 3.05

Figure 2: Properties of G114-loaded PLGA MPs. *(a) G114 pro-drug and its PSA-induced cleavage into the active toxin Leu-12ADT. (b) Physical and loading properties of G114-loaded MPs. To determine drug loading in the G114-MPs (mass of drug out of total MP mass) and the encapsulation efficiency (amount of drug encapsulated in MPs out of the initial amount of drug used in the MP production process), MPs were lysed overnight using NaOH-SDS, and G114 levels were measured using a microBCA assay). (c) SEM images of empty and G114-loaded MPs (x6500, scale bar is 5 μm). (d) Release kinetics of G114 from G114-loaded PLGA MPs (2.5mg MPs incubated in 10% FBS-supplemented 1mL MEM-α, media replaced at indicated time points and analyzed via LCMS).*

Average MP size was app. ~950nm **(Figure 2B-C)**, previously shown by our group to be suitable for successful internalization by MSCs[11,13,29]. Importantly, LCMS analysis demonstrated that prodrug-loaded MPs released significant amounts of the drug over time **(Figure 2D),** with 2.5 mg of G114-MPs releasing more than 70 µg of drug within 10 days, which is 20.56% of the total amount of encapsulated drug.

MSCs were incubated with different concentrations of G114-loaded PLGA MPs (G114 MPs) (0.025-0.5mg/mL for 15h), and assessed to confirm their intended use as cellular carriers for the MPs. As shown in **Figure 3A**, XTT analysis showed G114-MPs did not induce significant toxicity of MSCs at concentration of up to 0.5mg/mL. Based on this data and our previous work[36], 0.1 mg/mL was chosen as the working concentration for subsequent experiments.

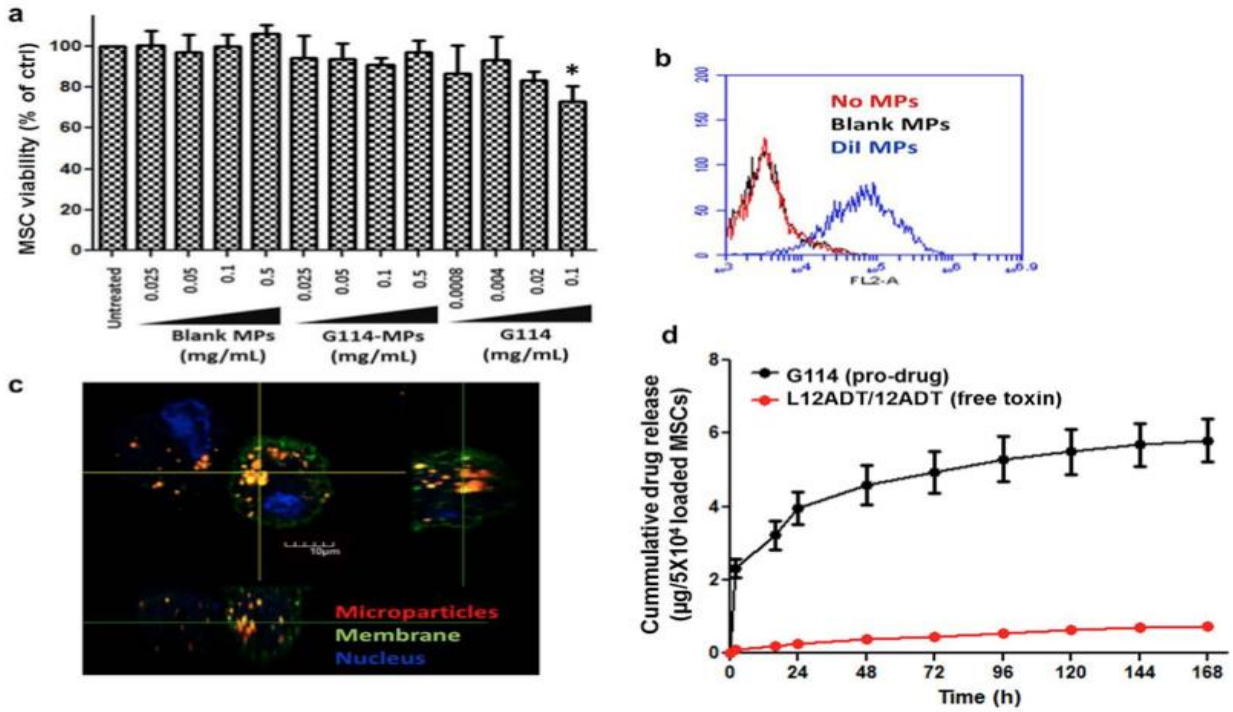


Figure 3: PLGA MPs are internalized by MSCs, followed by sustained release of intact G114 from G114-MP-loaded cells. *(a) MSCs were incubated with PLGA MPs (blank MPs or G114-MPs at 0.1mg/mL for 16h), followed by assessment of cell viability via XTT (*p<0.05 vs. untreated control, one-way ANOVA using Tukey's HSD, error bars represent SD). (b-c) DiI-loaded PLGA MPs, with similar properties to prodrug MPs, were incubated (0.1mg/mL) overnight with MSCs and flow cytometry analysis (b) and confocal microscopy (c) were performed to detect MP uptake and internalization by MSCs (red (DiI) - MPs, green (cholera toxin) - cell membrane, blue (Hoechst) - cell nucleus). (d) To assess drug release from G114 MP-loaded MSCs, MSCs (5x10⁴/well in a 6 well plate) were incubated with G114 MPs (0.1mg/mL MPs for 15h) and media (10% FBS-supplemented MEM-α) was collected at indicated time points and analyzed by LCMS for the presence of the intact G114 pro-drug and the free active toxins (L12ADT/12ADT).*

(SOW Task 2, subtasks 3-5) Using fabricated dye-encapsulating PLGA MPs with similar properties (~1µm in diameter), flow cytometry demonstrated association of those MPs with MSCs **(Figure 3B)**, and confocal microscopy confirmed that the MPs are fully internalized by MSCs **(Figure 3C)**. In addition, LCMS analysis of G114 MP- loaded MSCs revealed that G114 is released at significant levels **(Figure 3D).** A cumulative amount of over 5µg G114 was released from $5x10^4$ MP-loaded MSCs in 7 days, indicating the release of 116pg of G114 from each cell. More importantly, over 90% of the released drug retained its functional inactive pro-drug form

(G114) demonstrating that loading our delivery platform (MSCs) with G114 MPs enables release of the intact prodrug from the cells.

Furthermore, G114 released by G114 MP-loaded MSCs **(Figure 3D)** preferentially killed PSA-secreting cancer cells in the cell line LNCaP[23,28,30] *in-vitro* **(Figure 4A-B)**. Supernatant collected for 72h from MP-loaded MSCs applied on LNCaP cells was highly effective in inducing death of >90% of the PSA-secreting LNCaP prostate cancer cells within 72h **(Figure 4B)**. As a negative control, we used MDA-MB-231, a non-PSA-expressing breast cancer cell line **(Figure 4A)**. To further confirm this effect, we used a transwell co-culture between MP-loaded MSCs and LNCaP cells, simulating an *in-vivo* environment of direct proximity between the cancer and our therapeutic platform **(Figure 4C)**. G114-MP-loaded MSCs induced killing of ~70% of the PSA-secreting LNCaP cells within 72h, similar to the efficacy of free G114 (0.5 μM) applied directly on the cells **(Figure 4C)**. This data strongly suggests that G114 released from our MP-loaded MSCs is highly effective in killing PSA-secreting prostate cancer cells, demonstrating the promise of our cell-based therapeutic platform for potential use in prostate cancer therapy.

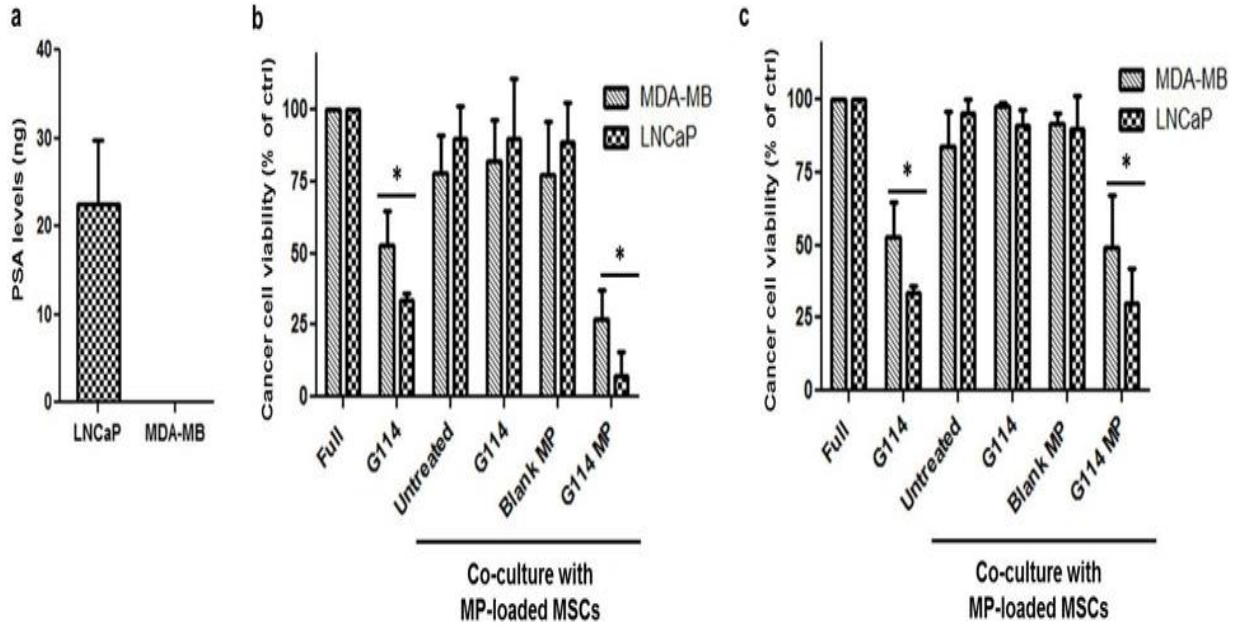
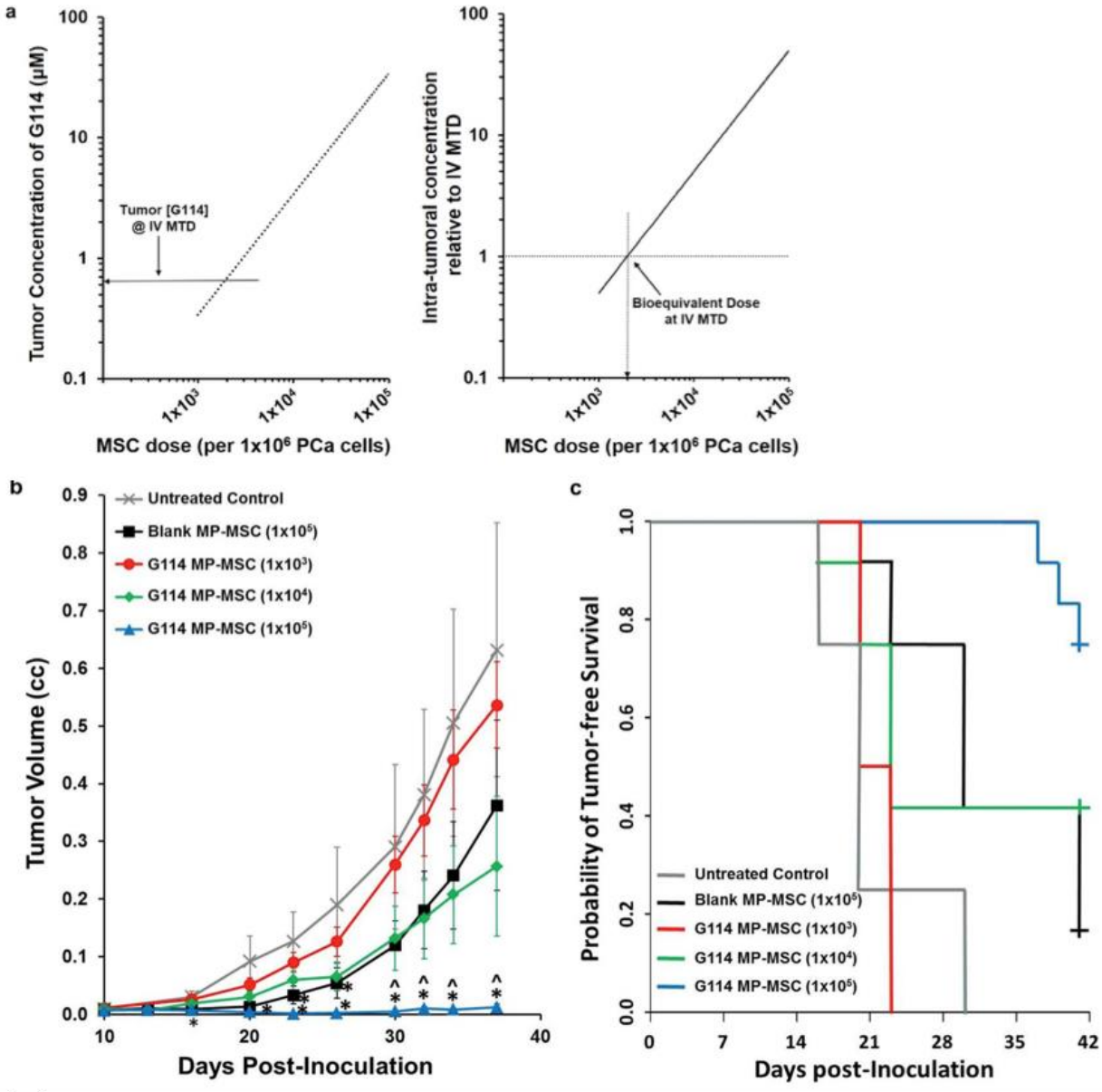


Figure 4: G114 released by G114 MP-loaded MSCs is effective in preferentially killing PSA-secreting PCa cells. *(a) PSA secretion by LNCaP PCa cells and MDA-MB-231 breast cancer cells (5x10^4 MSCs in 24h). (b) Following incubation of MSCs (5x10^4 cells) with G114 MPs (0.1mg/mL, 16hours), media was replaced, collected after 72h and then applied on LNCaP PCa cells and MDA-MB-231 breast cancer cells for 72h. Cancer cell viability was then tested via XTT (*p<0.05 vs. full media and vs. supernatant from untreated MSCs, one-way ANOVA using Tukey's HSD, error bars represent SD). (c) MP-loaded MSCs (5x10^4 cells) were co-cultured in a transwell system (0.4μm) in the presence of LNCaP or MDA-MB-231 cells (5x10^4 cells) for 72h, followed by XTT analysis to assess cancer cell viability (*p<0.05 vs. full media and vs. co-culture with untreated MSCs, one-way ANOVA using Tukey's HSD, error bars represent SD).*

(SOW Task 2, subtasks 6-7) Accordingly, we have predicted the intra-tumoral concentration of G114 as a function of the number of MSCs in a tumor comprising 1x10^6 PCa cells (**Figure 5A**). We considered a dose range of 1x10^3-1x10^5 G114 MP-loaded-MSCs to include the intra-tumoral concentration of G114 (i.e. 640nM) produced by the maximum tolerated intravenous dose (MTD) of G114 (i.e. 7 mg/kg)[21].



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Treatment Group		Median TTP (days)	TTP relative to Untreated Control	TTP relative to Blank MP-loaded MSC (1x10⁵)
Untreated Control		20	1.0	
Blank MP-loaded MSC	1x10⁵	30	1.5*	1.0
G114 MP-loaded MSC	1x10³	21.5	1.1	
	1x10⁴	23	1.2	
	1x10⁵	NE^	>2.1***	>1.4**

^ NE: not estimable. Median time was not reached because less than half of the mice developed tumors under this condition. *p = 0.012; **p = 0.003; ***p < 0.0001

Figure 5: In-vivo efficacy of G114 MP-loaded MSCs. *(a) The predicted bioequivalent dose of G114 MP-loaded MSCs vs. intravenously (IV) administered G114 at the maximum tolerated dose (MTD). (Right) Based upon the G114 release kinetics, the predicted intra-tumoral concentration of G114 delivered at different doses of MSCs co-inoculated with 1X10^6 PCa cells (in a 200µL volume) was calculated (ranging from 1x10^3 to 1x10^5 G114 MP-MSCs per 1x10^6 PCa cells). The intra-tumoral concentration of G114 when delivered intravenously (IV) at the MTD (i.e. 7 mg/kg) is 640 nM[21]. (Left) The predicted fold increase in the intra-tumoral concentration of G114 produced at these MSC doses over that achieved when G114 is delivered IV at the MTD. The bioequivalent dose (i.e. 640 nM) is predicted at a dose of 2x10^3 G114 MP-loaded MSCs per 1x10^6 PCa cells. 5- to 50-times more G114 is predicted to be delivered at the 1x10^4 and 1x10^5 MSC doses, respectively, than can be achieved at the systemically-administered MTD. (b) 1x10^6 CWR22 PCa cells were co-inoculated with blank- or G114 MP-loaded MSCs at the indicated doses in 200µl Matrigel/HBSS (50:50) into male athymic nude mice. Tumor volume was measured 3x/wk. Figure is the composite of two independent experiments (*p < 0.005 relative to untreated control, ^p < 0.05 relative to Blank MP-loaded MSC). (c) The probability of tumor-free survival over time. (d) G114 MP-loaded MSCs improve time to progression (TTP) in-vivo. The median number of days post-inoculation required to reach the TTP endpoint. TTP defined as ≥50% of animals in a group bearing a tumor (≥0.05cc). Fold improvement relative to the untreated control and the 1x10^5 dose of blank MP-loaded MSC groups were calculated (^NE: not estimable. Median time was not reached because less than half of the mice developed tumors under this condition. *p = 0.012; **p = 0.003; ***p < 0.0001).*

As shown in **Figure 5A**, the bioequivalent dose (i.e. 640 nM) is predicted at a dose of 2x10^3 G114 MP-loaded MSCs per 1x10^6 PCa cells. At 1x10^4 to 1x10^5 G114 MP-loaded MSCs, the engineered MSCs are predicted to deliver 5- to 50-times the intra-tumoral G114 concentration achieved at the systemic MTD (**Figure 5A**). To test these predictions and assess the impact of our cell-based platform on PCa xenograft growth, blank or G114 MP-loaded MSCs at doses ranging from 1x10^3 to 1x10^5 MSCs were co-inoculated subcutaneously with 1x10^6 CWR22 cells suspended in 200µL of matrigel into nude athymic mice (homogenous distribution of MSCs across the inoculate can be assumed since MSCs are mixed with CWR22 cells prior to co-inoculation). CWR22 xenografts were selected because they express a high amount of enzymatically active PSA[48]. G114-MP-loaded MSCs inhibit tumor growth of PCa xenografts in co-inoculation these *in-vivo* studies. As shown in **Figure 5B**, a dose-dependent inhibition of tumor growth was observed with near complete sterilization of the inoculum at the highest dose (i.e. 1x10^5 MSCs per 1x10^6 PCa cells). At this high dose, median time to progression (TTP) was increased >2.1-fold relative to untreated controls and >1.4-fold relative to a comparable dose of blank MP-loaded MSCs **(Figure 5C-D)**. Moreover, co-inoculation with G114-loaded MSCs loaded with a total amount of 11.6µg G114 at the maximal dose of 1x10^5 MSCs did not induce systemic toxicity as indicated by an insignificant effect on body weight (<5% change). Confirming our theoretical prediction **(Figure 5A)**, these data demonstrate that even at a co-inoculation dose of 1x10^4 G114 MP-loaded MSCs to 1x10^6 PCa cells, our cell-based platform displays an anti-tumor effect in PCa xenografts in-vivo.

4. Key Research Accomplishments

- **SOW Task 1 – ongoing.**
  - A Phase 0 pre-prostatectomy clinical trial (NCT01983709) is on-going. This study is designed to quantify the number of systemically infused allogeneic human bone marrow-derived MSCs to sites of PCa, which will provide the baseline conditions for the eventual clinical translation of the particle-in-a-cell platform.
- **SOW Task 2, subtask 1 – completed.**
  - G114 and PRX302 prodrug sources established.
- **SOW Task 2, subtask 2 – completed.**
  - Isolation and culture of hbMSCs optimized.

- **SOW Task 2, subtask 3 – completed.**
    - Successful encapsulation of prodrugs into PLGA MPs using a double emulsion protocol.
    - Optimization of a double emulsion protocol to achieve ~13% drug loading, ~88% encapsulation efficiency and ~950nm MP size.
- **SOW Task 2, subtask 4 – completed.**
    - Successful and complete internalization of G114-MPs into MSCs verified by flow cytometry and confocal microscopy.
    - Significant amount of prodrug released from MSCs over time. 90% of the released drug retains its functional prodrug form.
    - Released G114 within supernatant of MP-loaded MSCs preferentially kills PSA-secreting LNCaP prostate cancer cells over non-PSA-secreting MDA-MP-231 breast cancer cells.
    - Directly released G114 from MP-loaded MSCs within a transwell co-culture preferentially kills PSA-secreting LNCaP prostate cancer cells over non-PSA-secreting MDA-MP-231 breast cancer cells
- **SOW Task 2, subtask 5 – completed.**
    - Insignificant toxicity induced by G114-MPs on MSCs at concentrations of up to 0.5mg/mL
    - No effect on major MSC phenotypic markers observed
- **SOW Task 2, subtask 6-7 – completed/ongoing.**
    - Observed dose-dependent inhibition of tumor growth post co-inoculation of $1x10^3$ to $1x10^5$ G114 MP-loaded MSCs with $1x10^6$ PSA-secreting CWR22 cells.
    - At $1x10^5$ MSCs, systemic toxicity was not induced and median time to progression (TTP) was increased >2.1-fold relative to untreated controls and >1.4-fold relative to a comparable dose of blank MP-loaded MSCs.
    - Additional in vivo models, including soft tissue and bone CRPC models, to evaluate efficacy and quantify systemically-infused MSC homing are ongoing.
    - Multiple strategies to enhance MSC homing are also being explored.

5. Conclusion

We report the development of G114 MP-loaded MSCs as a cellular drug delivery platform. This cellular platform releases significant amounts of prodrug and selectively induces death of PSA-secreting PCa cells *in-vitro* as well as inhibits tumor growth following co-inoculation in CWR22 xenografts. This study provides proof-of-principle evidence that MSCs can be used as 'Trojan Horse' delivery vehicles to overcome limitations related to systemic delivery of cytotoxic agents. Importantly, this hypothesis is the subject of an ongoing Phase 0 pre-prostatectomy clinical trial (NCT01983709) designed to quantify the number of systemically infused allogeneic human bone marrow-derived MSCs to sites of PCa, which will provide the baseline bounded conditions for the eventual clinical translation of the platform. Overall, MP-loaded cells emerge as promising candidates for the systemic treatment of metastatic PCa.

We accomplished relatively high drug loading (>13%) and encapsulation efficiency (>88%). Furthermore, the encapsulated drug displayed sustained release from MPs and MP-loaded cells *in-vitro,* and is efficiently activated by PSA-secreting PCa cells, resulting in selective toxicity of G114 MP-loaded MSCs against PSA-expressing cells *in-vitro*. Moreover, co-inoculation of G114

MP-loaded MSCs with CWR22 PCa cells at a high effector-to-target ratio ($1x10^5$ G114 MP-loaded MSCs to $1x10^6$PCa cells) nearly eliminated tumor growth over the course of the assay and more than doubled TTP with dose-dependent responses observed at lower MSC/PCa ratios. The inhibition of tumor growth exhibited by blank-MP-loaded MSCs (which was still significantly lower than the impact of G11-MP-loaded MSCs at the same dose) may be attributed to PLGA-induced inflammatory activation of macrophages[49] or the previously reported anti-angiogenic effect of MSCs in other relevant cancer models[50,51]. Collectively, these data suggest that this MSC-based platform could deliver and activate therapeutically effective amounts of the pro-toxin if a sufficient number of cells infiltrated the tumor.

Looking ahead, future studies will focus on successfully translating this cellular platform for systemic targeted delivery of anti-cancer drugs to sites of PCa metastasis. A key aspect towards achieving therapeutic efficacy of this platform would be to maximize the number of systemically infused drug-doped MSCs that reach sites of PCa metastasis. To accomplish this goal, bioengineering the cells for improved targeting to tumor sites will be used. For instance, incubating MSCs in conditioned supernatant from irradiated cancer cells can enhance tumor targeting by upregulating the expression of multiple chemokine receptors, including CCR2[52]. HIF1-dependent upregulation of CXCL12 and CXCR4 in MSCs using preconditioning regimens including hypoxia, cobalt chloride and estrogen increase MSC trafficking to injured tissue[53–56]. Furthermore, we intend to explore multiple cell bioengineering approaches previously established in our laboratory such as surface chemical modification, mRNA transfection and small molecule pretreatment[57,58] to express homing ligands on the cell surface and maximize targeting of systemically infused cells to sites of PCa metastasis.

A second strategy to further enhance the efficacy of this cell-based platform as a systemic treatment is to use a more potent cytotoxic agent, thereby delivering therapeutically effective drug concentrations at lower ratios of infiltrating cells. An example of one such agent is the bacterial pore-forming pro-toxin, proaerolysin, which induces cell lysis at low picomolar concentrations[59-61]. We have previously engineered the pro-toxin to be selectively activated by PSA using site-directed mutagenesis to replace the wildtype furin activation site with a PSA-selective peptide sequence[61-62]. While PSA-activated proaerolysin has proven to be very effective as a local therapy for benign prostatic hyperplasia (BPH)[63,64], its usefulness as a systemic therapy is limited due to intrinsic limitations related to GPI-anchor binding and its mechanism of action, which prevent therapeutic concentrations from being achieved in target tissue following IV administration. These factors make it a promising candidate for further development as part of a cell-based delivery platform.

6. Publications, Abstracts and Presentations

"Attacking prostate cancer with a prodrug-doped cellular Trojan horse". Oren Levy, W. Nathaniel Brennen, Edward Han, David Marc Rosen, Juliet Musabeyezu, Helia Safaee, Sudhir Ranganath, Jessica Ngai, Martina Heinelt, Yuka Milton, Hao Wang, Neil Bhowmick, Samuel R. Denmeade, John T. Isaacs and Jeffrey M. Karp. **Under review in Biomaterials (find attached manuscript).**

"Rapid selection of mesenchymal stem and progenitor cells in primary prostate stromal cultures". Brennen WN, Kisteman LN, Isaacs JT. Prostate. 2016 Feb 2. [Epub ahead of print].

2015 A Mesenchymal Stem Cell (MSC)-based Macromolecular Prodrug Delivery Platform for the Treatment of Advanced Prostate Cancer.
22nd Annual Prostate Cancer Foundation (PCF) Scientific Retreat, Washington, D.C., USA.

2015 Mesenchymal Stem and Progenitor Cells in the Prostate: From Birth to Death and Potential Applications in Between.
Prostate Cancer Foundation Tumor Microenvironment/Immunology Working Group. Online.

2015 Mesenchymal Stem Cells (MSCs) in the Prostate from Birth to Death: Potential Marker of Lethal Disease?
8th Annual Multi-Institutional Prostate Cancer Program Retreat. Ft. Lauderdale, FL, USA.

2015 Mesenchymal Stem Cells (MSCs) in the Prostate from Birth to Death: Potential Marker of Lethal Disease?
10th Annual Johns Hopkins Prostate Research Day. Baltimore, MD, USA.

2015 Towards a cellular delivery platform for prostate cancer therapy
2015 Coffey-Holden Prostate Cancer Academy Meeting. La Jolla, CA, USA.

2015 Bio-engineering Strategies to Boost the Clinical Impact of Stem Cell-based Therapies
GTCbio - Analytics for Biologics meeting. Philadelphia, PA, USA

2015 Bio-engineering Strategies to Augment the Clinical Efficacy of Cell-based Therapies
Clinical and Surgical Translation of Stem Cells (SelectBio). San Diego, CA, USA.

7. Inventions, Patents and Licenses

None to date

8. Reportable Outcomes/Other Achievements

- Dr. Oren Levy (Instructor of Medicine at Prof. Karp's lab) received the BWH Department of Medicine Early Career Mentoring Award.
- Dr. Oren Levy received the 2015 William Randolph Hearst Foundation Young Investigator in Medicine Award.
- Dr. Oren Levy received The Charles A. King Trust Postdoctoral Research Fellowship.
- Dr. W. Nathaniel Brennen received the 2014 Clay and Lyn Hamlin Young Investigator Award, Prostate Cancer Foundation
- Dr. W. Nathaniel Brennen received a 2015 Faculty Recruitment Award, Maryland Cigarette Restitution Fund
- Dr. W. Nathaniel Brennen promoted to Assistant Professor, Prostate Cancer Program, Oncology, SKCCC at Johns Hopkins

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